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(54) Title: RECOMBINANT ANTIBODY AND METHOD

(57) Abstract

The present invention provides a humanised antibody molecule (HAM) having specificity for the TAG-72 antigen and having an antigen binding site wherein at least the complementarity determining regions (CDRs) of the variable domain are derived from the mouse monoclonal antibody B72.3 (B72.3 MAb) and the remaining immunoglobulin-derived parts of the HAM are derived from a human immunoglobulin and a process for its production.

each upper arm. The remainder of the structure, and
particularly the stem of the Y, mediates the effector
functions associated with immunoglobulins.

Natural immunoglobulins have been used in
assay, diagnosis and, to a more limited extent,
therapy. However, such uses, especially in therapy,
have been hindered by the polyclonal nature of
natural immunoglobulins. A significant step towards
the realisation of the potential of immunoglobulins
as therapeutic agents was the discovery of monoclonal
antibodies of defined specificity (1). However, most
MAbs are produced by fusions of rodent spleen cells
with rodent myeloma cells. They are therefore
essentially rodent proteins. There are very few
reports of the production of human MAbs.

Since most available MAbs are of rodent origin,
they are naturally antigenic in humans. Therefore,
the use of rodent MAbs as therapeutic agents in
humans is inherently limited by the fact that the
human subject will mount an immunological response to
the MAb and will either remove it entirely or at
least reduce its effectiveness.

There have therefore been made proposals for
making non-human MAbs less antigenic in humans. Such
techniques can be generically termed "humanizing"
MAbs. These techniques generally involve the use of
recombinant DNA technology to manipulate DNA
sequences encoding the polypeptide chains of the
antibody molecule.

Some early methods for carrying out such a
procedure are described in EP-A-0 171 496 (Res. Dev.
Corp. Japan), EP-A-0 173 494 (Stanford University),
EP-A-0 194 276 (Celltech Limited) and WO-A-8 702 671
(Int. Gen. Eng. Inc.). The Celltech application
discloses a process for preparing an

RECOMBINANT ANTIBODY AND METHOD

The present invention relates to a humanised
antibody molecule (HAM) having specificity for an
antigen present on certain malignant cells and to a
process for its production using recombinant DNA
technology.

In the present application, the term
"recombinant antibody molecule" (RAM) is used to
describe an antibody produced by any process
involving the use of recombinant DNA technology,
including any analogues of natural immunoglobulins or
their fragments. The term "humanised antibody
molecule" (HAM) is used to describe a molecule having
an antigen binding site derived from an
immunoglobulin from a non-human species, the
remaining immunoglobulin-derived parts of the
molecule being derived from a human immunoglobulin.
The antigen binding site may comprise either complete
variable domains fused onto constant domains or only
the complementarity determining regions grafted onto
appropriate framework regions in the variable
domains. The abbreviation "MAb" is used to indicate
a monoclonal antibody.

In the description, reference is made to a
number of publications by number. The publications
are listed in numerical order at the end of the
description.

Natural immunoglobulins have been known for
many years, as have the various fragments thereof,
such as the Fab, (Fab'): and Fc fragments, which
can be derived by enzymatic cleavage. Natural
immunoglobulins comprise a generally Y-shaped
molecule having an antigen-binding site at the end of

antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. It also shows the production of an antibody molecule comprising the variable domains of a mouse MAb, the CH1 and CL domains of a human immunoglobulin, and a non-immunoglobulin-derived protein in place of the Fc portion of the human immunoglobulin.

In an alternative approach, described in EP-A-87302620.7 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin using site directed mutagenesis using long oligonucleotides .

The earliest work on humanizing MAbs has been carried out based on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T cells respectively were humanized are shown by Verhoeyen et al. (2) and Reichmann et al. (3) .

It has been widely suggested that immunoglobulins, and in particular MAbs, could potentially be very useful in the diagnosis and treatment of cancer (4,5). There has therefore been much activity in trying to produce immunoglobulins or MAbs directed against tumour-specific antigens. So far, over one hundred MAbs directed against a variety of human carcinomas have been used in various aspects of tumour diagnosis or treatment (6).

There have been a number of papers published concerning the production of chimeric monoclonal antibodies recognising cell surface antigens. For instance, Sahagan et al. (7) disclose a genetically engineered murine/human chimeric antibody which

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retains specificity for a human tumour-associated
antigen. Also Nishimura et al. (8) disclose a
recombinant murine/human chimeric monoclonal antibody
specific for common acute lymphocytic leukemia
antigen.

According to the present invention, there is
provided a humanised antibody molecule (HAM) having
specificity for the TAG-72 antigen and having an
antigen binding site wherein at least the
complementarity determining regions (CDRs) of the
variable domain are derived from the mouse monoclonal
antibody B72.3 (B72.3 MAb) and the remaining
immunoglobulin-derived parts of the HAM are derived
from a human immunoglobulin.

The variable domains of the HAM may comprise
either the entire variable domains of the B72.3 MAb
or may comprise the framework regions of a human
variable domain having grafted thereon the CDRs of
the B72.3 MAb.

The B72.3 MAb is a mouse MAb of the type
IgG1-Kappa raised against a membrane-enriched extract
of a human liver metastatis of a breast carcinoma
(9). The B72.3 MAb has been extensively studied in a
number of laboratories. It has been shown to
recognise a tumour-associated glycoprotein TAG-72, a
mucin-like molecule with a molecular weight of
approximately 10^6 (10). Immunohistochemical
studies have demonstrated that the B72.3 MAb
recognises approximately 90% of colorectal
carcinomas, 85% of breast carcinomas and 95% of
ovarian carcinomas. However, it shows no significant
cross-reactivity with a wide spectrum of normal human
tissues (11 to 14).

It has surprisingly been found that humanizing
the B72.3 MAb does not adversely affect its binding

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activity, and this produces a HAM which is extremely useful in both therapy and diagnosis of certain carcinomas.

Preferably, the HAM of the present invention will be produced by recombinant DNA technology.

The HAM of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as the Fab or (Fab')₂ fragment; a light chain or heavy chain dimer; or any other molecule with the same specificity as the B72.3 antibody.

Alternatively, the HAM of the present invention may have attached to it an effector or reporter molecule. For instance, the HAM may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce a HAM in which the Fc fragment or CH3 domain of a complete antibody molecule has been replaced by an enzyme or toxin molecule.

The remainder of the HAM may be derived from any suitable human immunoglobulin. However, it need not comprise only protein sequences from the human immunoglobulin. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a polypeptide effector or reporter molecule.

According to a second aspect of the present invention, there is provided a process for producing the HAM of the first aspect of the invention, which process comprises:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy

or light chain wherein at least the CDRs of the
variable domain are derived from the B72.3 MAb and
the remaining immunoglobulin-derived parts of the
antibody chain are derived from a human
immunoglobulin;

(b) producing in an expression vector an
operon having a DNA sequence which encodes a
complementary antibody light or heavy chain wherein
at least the CDRs of the variable domain are derived
from the B72.3 MAb and the remaining
immunoglobulin-derived parts of the antibody chain
are derived from a human immunoglobulin;

(c) transfecting a host cell with the or each
vector; and

(d) culturing the transfected cell line to
produce the HAM.

The cell line may be transfected with two
vectors, the first vector containing an operon
encoding a light chain-derived polypeptide and the
second vector containing an operon encoding a heavy
chain-derived polypeptide. Preferably, the vectors
are identical except in so far as the coding
sequences and selectable markers are concerned so as
to ensure as far as possible that each polypeptide
chain is equally expressed.

Alternatively, a single vector may be used, the
vector including the sequences encoding both light
chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light
and heavy chains may comprise cDNA or genomic DNA or
both. However, it is preferred that the DNA sequence
encoding the heavy or light chain comprises at least
partially genomic DNA. Most preferably, the heavy or
light chain encoding sequence comprises a fusion of
cDNA and genomic DNA.

The host cell used to express the HAM of the
present invention is preferably a eukaryotic cell,
most preferably a mammalian cell, such as a CHO cell
or a myeloid cell. It has been found, surprisingly,
that the use of cDNA/genomic D.A fusions for the
heavy or light chain coding sequences leads to
enhanced production of the HAM of the present
invention in non-myeloid mammalian cells. Thus, an
important aspect of the invention is the use of such
fusions in non-myeloid mammalian cells in order to
express the HAM.

The present invention also includes cloning and
expression vectors and transfected cell lines used in
the process of the invention, therapeutic and
diagnostic compositions containing the HAM of the
invention and uses of such compositions in therapy
and diagnosis.

The general methods by which the vectors may be
constructed, transfection methods and culture methods
are well known per se and form no part of the
invention. Such methods are shown, for instance, in
references 15 and 16.

The present invention is now described, by way
of example only, with reference to the accompanying
drawings, in which:-

Figure 1 shows the DNA sequences encoding the
unprocessed variable regions of the B72.3 MAb
obtained by sequencing the cDNA clones pBH41 and
pBL52. Panel A shows the sequence coding for the VH
region and the predicted amino acid sequence. Panel
B shows the sequence coding for the VL region and the
first 21 residues of the CL region, together with the
predicted amino acid sequence. The points of fusion
with the human C regions are indicated with arrows.

The putative sites of cleavage of the signal peptide are indicated by open triangles. The numbers refer to the nucleotides in the original cDNA clones;

Figure 2 is a schematic diagram of the construction by site directed mutagenesis, restriction and ligation of the chimaeric heavy chain gene;

Figure 3 is a schematic diagram of the construction by partial restriction and ligation of the chimaeric light chain gene;

(In Figures 2 and 3, coding sequences are shown as boxes, dark for the variable regions and light for the constant regions. Restriction enzymes are abbreviated as follows: EcoRI=E; BglII=B; HindIII=H; MboII=M; HpaI=Hp; and ScaI=Sc. Dotted lines indicate the continuation of the sequence into vector or constant region DNA.)

Figure 4 is a schematic diagram of the hCMV expression vector and the four alternative cDNA or gene constructs inserted into the EcoRI site. The chimaeric heavy chain gene was inserted using a BamHI-EcoRI oligonucleotide adapter. Coding sequences are represented by boxes, dark for the variable regions and light for the constant regions. The direction of transcription is indicated with an arrow;

Figure 5 shows an ELISA analysis of COS-cell transfectant supernatants. The level of antigen-binding capacity in the supernatant of COS-cell transfectants was analysed as described later. Dilution curves were plotted out against the optical density of the colour change. Different antibodies were used to recognise mouse or human epitopes, and consequently the antigen-binding levels for each curve are not strictly comparable. Each

curve represents a co-transfection, as follows:
$\triangle$ mouse heavy chain, mouse light chain; $\blacktriangle$ mouse
heavy chain, chimaeric light chain; $\square$ chimaeric heavy
chain, mouse light chain; $\blacksquare$ chimaeric heavy chain,
chimaeric light chain;

Figure 6 shows SDS-PAGE analysis in a reducing
gel of immunoprecipitations from the supernatants of
transfected COS-cells. The DNA used for the
transfection was as follows: Lane 1, mouse light
chain alone; Lane 2, mouse light chain, mouse heavy
chain; Lane 3, mouse light chain, chimaeric heavy
chain; Lane 4, chimaeric light chain alone; Lane 5,
chimaeric light chain, mouse heavy chain; and Lane 6,
chimaeric light chain, chimaeric heavy chain. The
antibodies used for the immunoprecipitations were:
Lanes 1-3, rabbit anti-mouse $F(ab')_2$; Lanes 4-6,
rabbit anti-human $F(ab')_2$;

Figure 7 shows SDS-PAGE analysis of
immunoprecipitations from the supernatants of
transfected COS-cells, under non-reducing (lanes
1-3), and reducing (lanes 4-6) conditions. The DNA
used for transfection was as follows: lanes 1 and 4,
chimaeric light chain clone; lanes 2 and 5, chimaeric
light chain, mouse heavy chain; lanes 3 and 6,
chimaeric light chain, chimaeric heavy chain. The
antibody used for the immunoprecipitation in each
case was rabbit anti-human $F(ab')_2$;

Figure 8 shows SDS-PAGE analysis on a reducing
gel of immunoprecipitations from the supernatants of
transfected COS-cells, grown and labelled in the
absence (lanes 1 and 3), and the presence (lanes 2
and 4) of tunicamycin. The DNA used for the
transfections was as follows: lanes 1 and 2,
chimaeric light chain clone; and lanes 3 and 4,
chimaeric light chain and chimaeric heavy chain. The

antibody used for immunoprecipitation in each case was rabbit anti-human F(ab')₂ ;

Figure 9 shows reducing and non-reducing SDS-PAGE gels of chimeric B72.3 produced by CHO cells;

Figure 10 shows a twc dimensional SDS-PAGE gel of chimaeric B72.3 produced by CHO cells;

Figure 11 shows a time course study of tumour labelling using B72.3 antibodies;

Figure 12 shows the tissue/tumour ratio of the B72.3 antibodies; and

Figure 13 shows the construction of plasmid TR002

EXAMPLE 1

Molecular cloning and sequencing of the B72.3 heavy and light chain cDNAs.

Polyadenylated RNA was isolated from the B72.3 hybridoma cell line using the guanidinium isothiocyanate/caesium chloride method (15). Double stranded cDNA was synthesised (17) and a cDNA library was constructed in bacteriophage λgt 10 vector using EcoRI linkers (18). Two screening probes were synthesised, complementary to mouse immunoglobulin heavy and light chain constant regions. The heavy chain probe was a 19 mer complementary to residues 115-133 in the CH1 domain of the mouse γ1 sequence (19). The light chain probe was a 20 mer complementary to residues 4658-4677 of the genomic mouse CK sequence (20). The probes were radio-labelled at the 5' terminus with [γ³²P] ATP using T4 polynucleotide kinase (Amersham International) and used to screen the cDNA library.

Clones which contained the complete leader, variable and constant regions of both the heavy and

light chains were isolated. The EcoRI cDNA inserts
were subcloned into M13mp8 vectors for sequencing
(21), generating a heavy chain clone, designated
pBH41, and a light chain clone, designated pBL52.
Nucleotide sequence analysis was carried out
according to the chain termination procedure (22).

The 980 base pair EcoRI insert in pBL52 was
fully sequenced (22). The EcoRI insert in pBH41 was
shown to comprise approximately 1700 base pairs by
agarose gel electrophoresis. The variable domain and
the 5' region of the CH1 domain were sequenced, as
was the 3' end of the clone to confirm the presence
of the correct mouse δ1 termination sequences. The
DNA and predicted amino acid sequences for the
unprocessed variable regions of pBH41 and pBL52 are
shown in Figure 1. Examination of the derived amino
acid sequence revealed considerable homology with
other characterised immunoglobulin genes, and enabled
the extent of the leader, variable and constant
domains to be accurately determined. In addition,
MAb B72.3 was confirmed to be an IgG1 K antibody, as
previously reported (9).

Construction of the Chimaeric Mouse-Human Heavy Chain Clone

A genomic clone containing sequences coding for
the human Cδ4 region was isolated as a HindIII
fragment from the cosmid COS Ig8 (23) and then cloned
via pAT153 into M13tg130 as an EcoRI-BamHI fragment
to form pJA78. Following DNA sequence analysis, an
18 mer oligonucleotide was synthesised and site
specific mutagenesis was performed to convert a C
residue to an A residue, thereby generating a novel
HindIII site at the start of the CH1 exon, to yield
pJA91.

Site directed mutagenesis was performed (24) using EcoRI- and BglII-cut M13mp18 to generate a gapped duplex with the relevant phage template. DNA was transformed into E. coli HB2154 and resultant transformants were propagated on E. coli HB2151 (Anglian Biotechnology Ltd) as described in the protocols provided. All mutations were sequenced using the chain termination procedure (22). All sequenced fragments were subsequently recloned into other vectors in order to exclude the possibility of secondary mutations which may have occurred during the mutagenesis procedure.

The VH domain from the B72.3 heavy chain cDNA, cloned in M13mp9 as pBH41, was isolated as an EcoRI-BglI fragment and introduced into the EcoRI-HindIII sites of pJA91 in conjunction with a 32 base pair BglI-HindIII adaptor to yield pJA93. The product was therefore a chimaeric immunoglobulin heavy chain gene containing a variable region derived from a mouse cDNA clone fused to a sequence, comprising the CH1, H, CH2 and CH3 domains separated by introns, derived from a human genomic clone. The accuracy of the variable/constant region junction was confirmed by nucleotide sequence analysis. A schematic diagram of details of the construction is given in Figure 2. The γ4 constant region was selected as it possesses a limited repertoire of effector functions, but does bind to staphylococcal protein A, a potentially useful reagent for purification.

Construction of the Chimaeric Mouse-Human Light Chain Gene

The mouse light chain cDNA clone, pBL52, contains a cutting site for MboII 18 base pairs

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downstream from the junction of the variable and
constant domains. Due to sequence homology between
the mouse and human CK genes, an identical cutting
site exists in the latter gene (25) and use of this
site provides a method of fusing the mouse variable
and human constant domains. Partial digestion of the
EcoRI fragment containing the mouse cDNA clone with
MboII generated a 416 base pair EcoRI-MboII fragment
with a single residue overhang. A genomic clone,
comprising an M13-derived vector containing the human
C-kappa gene on a PstI-HindIII fragment was digested
with FokI. A 395 base pair fragment containing the
majority of C-kappa was cloned into pAT153 using
EcoRI linkers to form pNW200. Digestion of a 945
base pair ScaI-HindIII fragment from pNW200 with
MboII generated a 374 base pair MboII-HindIII
fragment, which could anneal with and be ligated to
the 416 base pair EcoRI-MboII fragment described
above. The two fragments were ligated into a pSP64
vector linearised with EcoRI and HindIII, and used to
transform competent E. coli HB101. The
variable/constant region junction was sequenced in
order to confirm the correct fusion. The
construction is outlined schematically in Figure 3.

Construction of Expression Vectors for Transient Expression in COS Cells

The heavy and light chain chimaeric genes, as
well as the mouse heavy and light chain cDNA clones,
were inserted separately into the unique EcoRI site
of plasmid pEE6 (27). The light chain encoding
plasmid was designated EE6.cL.neo. For the chimaeric
heavy chain, this was accomplished by using an
oligonucleotide adapter to change the 3' BamHI site

to an EcoRI site to give an EcoRI fragment for
cloning. The heavy chain encoding plasmid was
designated EE6.cH.gpt. This plasmid contains the
strong promoter/enhancer and transcriptional control
element from the human cytomegalovirus (hCMV)
inserted into a unique HindIII site upstream of the
EcoRI site. In addition, an SV40 origin of
replication site is provided by the SV40 early
promoter which drives a selectable marker gene,
either a neomycin-resistance gene (neo) for light
chain genes or a guanine phosphoribosyl transferase
gene (gpt) for heavy chain genes. inserted into a
unique BamHI site. The plasmid also contains an
ampicillin-resistance gene allowing selection and
propagation in bacterial hosts. The structures of
the expression vector and immunoglobulin gene insert;
are shown schematically in Figure 4.

Transfections and ELISA Analysis of Antibody Production

The four expression constructs described above
were used singly or in heavy/light chain gene pairs
to transfect COS-1 cells (26). The cells were left
to incubate in DNA-DEAE dextran solution for six
hours, then shocked for two minutes with 10% DMSO in
HEPES-buffered saline. The cells were washed and
incubated in medium containing 10% foetal calf serum
for 72 hours.

Following incubation at 37°C for 72 hours the
cell supernatants and lysates were analysed by ELISA
for heavy and light chain production and binding of
antigen.

The medium (500 µl per 10^5 cells) was removed
for ELISA analysis. Cell lysates were prepared by

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lysis of 10^5 cells in 500 µl 1% Triton X-100, 0.5%
deoxycholate, 0.1% SDS, 0.01M sodium phosphate pH
7.5, 0.1M sodium chloride and 0.001M EDTA. Lysates
and conditioned medium were centrifuged for 5 minutes
in an Eppendorf centrifuge to remove nuclei and cell
debris, and stored at 4°C before analysis.

Microtitre plates were coated with 0.25 µg per
well of sheep or goat antibody reactive against
either human or mouse specific epitopes on the heavy
or light chains. Supernatants or lysates from
transfected COS cells were diluted 1:2 or 1:4
respectively in sample conjugate buffer containing
0.1M Tris-HCl pH 7.0, 0.1M sodium chloride, 0.02%
Tween 20 and 0.2% casein. 100 µl of each diluted
sample were added to each well and incubated for 1
hour at room temperature with gentle agitation.
Following washing six times with wash buffer
(phosphate buffered saline containing 0.2% Tween 20,
pH 7.2), 100 µl of 1:5000 dilution of standard
horseradish peroxidase - conjugated antibody reactive
against either human or mouse specific epitopes were
added per well. The plates were incubated for 1 hour
at room temperature, and then washed six times with
wash buffer. 100 µl of substrate buffer containing
0.1 mg/ml tetramethylbenzidine (TMB), 0.1M sodium
citrate, pH 6.0 and 0.005% H_2O_2 were added to
each well to generate a colour change. The reaction
was terminated after 2-3 minutes by adjusting the
solution to pH 1.0 with 1.5M sulphuric acid. The
optical density was determined at 450nm for each well
by measurement in a Dynatech laboratories MR600
microplate reader. Standard curves were generated
using known concentrations of the appropriate human
or mouse immunoglobulins.

Antigen binding assays were performed in an
analogous manner. Microtitre plates were coated with
0.25 µg per well of purified TAG-72 antigen (6)
obtained from human patients or from tumour
xenografts implanted in nude mice (both gifts of J.
Schlom, NCI). Following washing six times in wash
buffer, samples from COS-cell transfections were
added as previously, and the same subsequent
procedures carried out, using goat anti-mouse or
human $F(ab')_2$ linked to HRP as the second antibody.

A number of assay systems using different
capture antibodies were developed and
cross-correlated to investigate the potential
products of each transfection. In all cases, mouse
light chain and chimaeric light chain were detected
in the supernatants and lysates of appropriately
transfected cells. However heavy chains were only
detected in the supernatants when co-transfected with
light chain. A low level of heavy chain was detected
in the cell lysate in each case, supporting a
suggestion of inhibition of heavy chain secretion in
the absence of light chain.

Assembly assays, which detect the presence of
associated polypeptide chains, demonstrated the
formation of multimers containing at least one heavy
and one light chain when both genes were
co-transfected. Mouse genes and chimaeric genes
appeared equally capable of assembly and formation of
hybrid molecules.

Antigen binding analysis (see above)
demonstrated that the mouse heavy and light chain
co-transfections generated an antibody molecule
capable of recognising antigen. Replacement of the
mouse gene for either chain with the appropriate
chimaeric gene led to the production of a hybrid

molecule with antigen binding specificity in the
ELISA assays. Finally, transfection of the COS cells
with both the chimaeric heavy and light chain genes
generated a complete chimaeric antibody molecule with
antigen binding specificity. The ELISA data from one
experiment are presented in Figure 5. These
experiments demonstrate that "humanisation" of the
antibody molecule does not have a significant effect
on its antigen recognition capability.

Immunoprecipitation of Antibody Molecules from
Biosynthetically Labelled COS-Cell Transfectants

Preliminary experiments suggested that there
was little expression from the transfected DNA in the
initial 24 hours. Accordingly following
transfection, COS cells were allowed to recover for
24 hours in DMEM containing 10% foetal calf serum.
The medium was then replaced with methionine-free
DMEM, to which [^{35}S] methionine (NEN) had been
added at 200 µCi/ml. The cells were metabolically
labelled for 48 hours, and conditioned media and cell
lysates prepared as previously.

Analysis by reducing SDS-PAGE of aliquots of
COS cell supernatant demonstrated the appearance of
labelled immunoglobulin protein without further
purification, while use of Protein A-Sepharose was
shown to selectively precipitate whole antibody, but
not light chain alone, from the COS-cell supernatant.

Further analysis of the assembly and secretion
of antibody molecules was performed by
immunoprecipitation using anti-human F(ab')$_2$ and
anti-human C-kappa anti-sera bound to Protein
A-Sepharose. Affinity-purified rabbit antibodies
against human IgG F(ab')$_2$ and human K chain were

used for immunoprecipitations, following coupling to
Protein A - Sepharose. Both cytoplasmic and secreted
antibodies were analysed on an SDS-10% PAGE system
under reducing and non-reducing conditions. The gel
was treated with an autoradiography enhancer, dried
and exposed to Fuji RX film. The results are shown
in Figure 6.

Both antisera immunoprecipitated proteins with
an apparent molecular weight of 55K and 28K, which
coincided with the positions of the Coomassie-stained
immunoprecipitating heavy and light immunoglobulin
chains respectively. The use of the latter antisera
demonstrated that light chain is found associated
with heavy chain in the COS-cell supernatants. A
comparison of immunoprecipitations analysed by
reducing and non-reducing SDS-PAGE (See Figure 7)
suggests that the heavy and light chains are
assembled as the correct tetrameric molecule. In
addition there is evidence for the secretion of free
light chain dimers and partially assembled heavy and
light chain multimers.

Due to the presence of secondary structure
caused by disulphide bonds, the mobility of the
immunoglobulin chains is different in the two
systems. In order to analyse the presence and
potential role of glycosylation, COS cells were
treated with tunicamycin, at the same time as the
radioactive label was added. To inhibit N-linked
glycosylation, COS cells were grown in medium
containing 10 µg/ml tunicamycin diluted from a stock
solution. To ensure that the pool of lipid-linked
oligosaccharide was depleted, the cells were
maintained in the tunicamycin-containing medium for 2
hours prior to addition of radioactive label.

Following immunoprecipitation the protein
products were analysed by SDS-PAGE, as shown in
Figure 8. It is clear from the decrease in apparent
molecular weight, from 55K to 52K, that the chimaeric
heavy chain, but not the light chain, undergoes
N-linked glycosylation. When the glycosylation is
inhibited, the protein is still secreted, although
the level of expression appears to be decreased.

These results demonstrate that each of the
immunoglobulin genes is correctly transcribed and
translated. The two mouse genes and the chimaeric
light chain are cDNA-like, while the chimaeric heavy
chain gene possesses characteristics of both cDNA and
genomic DNA. Both types of construct appear to be
expressed at a similar level and with similar
fidelity. It is clear therefore that transcript
splicing occurs where necessary, but it is not an
obligatory requirement for correct expression of
immunoglobulin genes in COS-cells.

The expressed heavy and light chains associate
in the correct manner, presumably limited by the
availability of free polypeptide chain. Mouse and
human polypeptide sequences appear interchangeable in
the association of heavy and light chains. The
product is an assembled tetrameric antibody molecule,
which is expressed at a high level, glycosylated and
secreted into the culture medium.

Development of Stable Cell Lines in CHO Cells

Stable Light Chain Producing Cell Line

Chinese hamster ovary (CHO-K1) cells were grown
in attached culture in Dulbecco's Modified Eagles
Medium (DMEM) containing 10% foetal calf serum (FCS),

non-essential amino acids (NEAA) and glutamine
(2mM). A confluent culture was trypsinised, the
cells washed once in phosphate buffered saline (PBS)
and resuspended at 10⁷ cells/ml.

DNA from plasmid EE6.cL.neo, comprising the
chimaeric light chain gene expressed from the HCMV
promoter, was digested with SalI to generate linear
molecules and then ethanol precipitated. The
precipitate was resuspended in PBS and 40 µg of DNA
was added to 10⁷ CHO-K1 cells in buffer at 4°C.
The DNA was introduced into the cells by
electroporation, in which the cell suspension and DNA
were treated with two pulses of 2000 volts. After
electroporation the cells were returned to 4°C for
10 min prior to plating out at a density of 5 x 10⁵
cells per 90mm Petri dish in DMEM growth medium
containing supplements.

Following incubation at 37°C overnight,
selection for introduced DNA was applied by adding
G418 to a final concentration of 1mg/ml. Resistant
colonies were observed after 10-14 days incubation in
selective medium.

Resistant colonies were picked from the
transformation plates using filter paper squares
soaked in trypsin solution and transferred into
individual wells of 24 well tissue culture plates.
The culture medium from the wells was assayed for the
presence of chimaeric light chain using an ELISA
assay and cell lines secreting light chain were
identified. Lines producing light chain at levels
between 100ng/ml and 16 µg/ml were identified. Those
producing the highest amounts were cloned out by the
limiting dilution method. One such clone, cL18, was
used for subsequent studies.

Stable Chimaeric Antibody Producing Cell Line

DNA from plasmid EE6.cH.gpt (also designated as JA96), comprising the chimaeric heavy chain gene expressed from the HCMV promoter, was digested with SalI to generate linear molecules and then ethanol prepicitated. CHO-cL18 cells producing chimaeric light chain were prepared for electroporation as described above, and the JA96 DNA (40 µg per 10^7 cells) was introduced by electroporation. Following overnight incubation in non-selective medium, selection for resistance to mycophenolic acid was applied. Selective medium comprised DMEM, 10% FCS, NEAA, glutamine, xanthine, hypoxanthine, thymine and mycophenolic acid (10 µg/ml). Resistant colonies were detected after 10-14 days and these were picked into 24 well plates as described above. Antibody producing cell lines were identified using an antigen binding assay based on the antigen TAG-72 recognised by the antibody B72.3. Cell lines producing antibody at levels ranging from 0.1-40 µg/ml were isolated. Two of these cell lines, F6 and F11, were used in further studies.

Purification of Chimaeric Antibody

Chimaeric B72.3 was purified from CHO cell culture supernatant by affinity chromatography using Protein-A Sepharose and ion-exchange chromatography. Cell culture supernatant was adjusted to pH 8.8 with sodium glycinate (0.2M) and applied to a column of Protein-A Sepharose pre-equilibrated with glycine/glycinate buffer at pH 8.8 (50mM). After sample loading, the column was washed with equilibration buffer and the antibody eluted with a

gradient of decreasing pH made up of disodium
hydrogen phosphate (0.2M) and citric acid (0.1M).
Fractions containing chimaeric antibody were pooled,
dialyzed into 50 mM phosphate buffer pH 8.0 and then
applied to a column of DEAE-Sepharose
pre-equilibrated with 50mM phosphate buffer pH 8.0.
The column was washed with equilibration buffer and
elution of antibody achieved with a linear gradient
of sodium chloride from 0.0 to 0.2M. Purified
antibody was then dialyzed into an appropriate buffer
for the intended use, e.g. PBS for animal studies,
and concentrated by ultrafiltration. Typical yields
of chimaeric antibody were 20 mg per litre of
starting supernatant.

    Purity and assembly of the chimaeric antibody
was tested by SDS-polyacrylamide gel electrophoresis
(PAGE), both reducing and non-reducing (Fig 9), and
by HPLC gel filtration.

    N-terminal amino acid sequencing of the
antibody revealed a single sequence encoding light
chain, which corresponded exactly to the expected
amino acid sequence deduced from the DNA sequence.
The heavy chain was N-terminally blocked and not
amenable to amino acid sequencing. Antigen binding
activity was demonstrated in an ELISA format assay.

    Chimaeric B72.3 made in COS or CHO cells
contains a proportion of material (10-20%) which
fails to form an inter-heavy chain disulphide bridge
but otherwise assembles correctly into 150kD
molecules containing two heavy and two light chains.
This material is present in antibody when secreted
from the cell and co-purifies with antibody in which
the inter-heavy chain disulphide bridges have
formed. This appears to be a common property of
human IgG4 molecules and occurs with all molecules of

this type analysed to date, including a mouse-human
IgG4 chimaeric anti-NP antibody and two different
IgG4 myeloma proteins.

On non-reducing SDS-polyacrylamide gel
electrophoresis of chimaeric B72.3, two bands are
seen, one at the expected size of 150 kD and one of
about 80 kD (Fig. 9) Both of these bands contain
intact heavy and light chains as shown by
non-reducing/reducing two dimensional SDS-PAGE (Fig.
10). Reducing SDS-PAGE shows only intact heavy and
light chain (Fig. 9). Native (non SDS)
electrophoresis and HPLC gel filtration show only one
species corresponding to 150 kD material. Thus the
about 80 kD band seen on non-reducing SDS-PAGE
represents materials with a molecular weight of 150
kD in solution. The two halves of the molecule are
only separated when other inter-heavy chain
interactions are disrupted, e.g. when run on
non-reducing SDS-PAGE

Efficacy Studies

The chimaeric B72.3 antibody is capable of
being used to advantage in a number of
circumstances. For example, after suitable labelling
by radioisotopes or other detection procedure, the
antibody can be demonstrated to locate and bind in
vivo to solid tumours where some or all of the tumour
cells express the specific antigen TAG-72. The
experiment described below is one example of the
ability of the chimaeric antibody to locate human
tumour cells bearing the specific antigen, in this
case in a nude mouse model system.

Chimaeric B72.3 and mouse B72.3 antibodies were
radioactively labelled with ^{125}I by the Chloramine

T method to an approximate specific activity of
5 μCi/μg. Groups of 4 female nude mice bearing
subcutaneous LS174T xenografts on the flank were
injected intravenously with 100 μCi of either
Chimaeric B72.3 or mouse B,2.3 in 0.1ml PBS. Groups
of animals were sacrificed at intervals for the
collection of tissue samples, which were weighed,
dissolved in 7M potassium hydroxide and counted in an
LKB model 1270 "Rackbeta" counter. Tissue uptake was
calculated as the mean percentage of injected dose
per gram of tissue from a group of four animals.

Fig. 11 shows a time course study of the mouse
and chimaeric antibodies and demonstrates clearance
of the antibodies from the blood pool and uptake at
the tumour site. The chimaeric antibody appears to
clear somewhat faster from the blood pool but locates
to the tumour adequately with approximately the same
profile as the mouse antibody. This sample data
suggests that the novel engineered antibody is
functional in vivo.

Fig. 12 shows the tumour to tissue ratio at 24,
48 and 168 h. It can be seen that tumour/tissue
ratios increase with time and that in this model
system the chimaeric antibody has a superior
tumour/tissue ratio compared to the mouse antibody.

EXAMPLE 2

Chimaeric B72.3 - Other IgG Isotypes

Construction of Chimaeric Antibody Genes

Assembly of Chimaeric Antibody Genes

Genomic DNA sequences containing the human
IgG1, 2 and 3 genes were isolated from larger DNA

inserts in phage λ and were introduced into phage
M13 via pJA103 which contains the human IgG4 gene
with a HindIII site at the 5' end of the CH1 exon and
a BamHI 3' to the CH3 exon. The M13 vector is
M13tg130 which has two amber mutations in essential
genes and is therefore suitable for high efficiency
site-directed mutagenesis experiments using the
procedures described earlier. A HindIII site was
introduced at the 5' end of the CH1 exon in each
isotype gene to give pRB11 (IgG1), pRB14 (IgG2) and
pRB16 (IgG3). SalI and BglII sites were also
introduced into pRB11 towards the 3' end of the CH1
exon and towards the 3' end of the intron following
the CH1 exon respectively. The isotypes were then
reisolated as HindIII-BamHI fragments and sub-cloned
into pAT153 to give RB18 (IgG1), RB26 (IgG2), and
RB20 (IgG3). The B72.3 VH DNA sequence was isolated
and was ligated to the linking oligonucleotide which
was used earlier to make the IgG4 chimaeric heavy
chain gene so as to give an EcoRI-HindIII VH
fragment. This fragment was ligated to the human
IgG1 HindIII-BamHI containing fragment of RB18 and
cloned in pAT153 to give pRB22.
To construct the chimaeric B72.3 VH, the VH fragment
described above was ligated to the HindIII-BamHI
fragment of pRB26 and recloned in pAT153 to give
pRB27.

To construct the chimaeirc B72.3 VH/IgG3 gene,
the VH fragment described above was ligated to the
HindIII-BamHI fragment of pRB20 and recloned in
pAT153 to give pRB23.

Assembly of Genes in Expression Vectors

The chimaeric genes were isolated as
EcoRI-BamHI fragments from pRB22, 27 and 23 described

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above and cloned between the EcoRI and BclI sites of
JA96, the B72.3 IgG4 chimaeric heavy chain expression
vector, thus replacing the IgG4 chimaeric gene. The
resultant chimaeric expression plasmids were named
RB24 (IgG1 chimaera), RB28 (IgG2 chimaera) and RB25
(IgG3 chimaera)

     Demonstration of production, assembly and
activity were performed as in Example 1.

                       EXAMPLE 3

Chimaeric B72.3 IgG4 F(ab')2

Construction of F(ab') Heavy Chain Gene

Assembly of F(ab') Gene

     pJA79 is an M12tg130 vector which contains the
human IgG4 heavy chain gene modified so that the
sequence from the first nucleotide after the last
codon of the hinge exon to the last nucleotide of the
CH3 domain inclusive has been removed by
oligonucleotide directed site specific deletion.
The hinge and 3' untranslated region and part of the
M13 sequence can be isolated as a 1.1 kbp BglII
fragment. This fragment can be used to replace the
analogous fragment in the full length B72.3/IgG4
chimaeric heavy chain gene clone pJA93 to give
plasmid JA94 which therefore contains a chimaeric
gene potentially capable of being expressed to
produce a B72.3 IgG4 chimaeric F(ab') heavy chain
protein.

Assembly of Gene in Expression Vector

Plasmid pJA94 described above was used to recover the F(ab') gene as an EcoRI-BamHI 1475 bp fragment. This fragment was cloned into the unique EcoRI site of the pEE6 expression vector using a BamHI to EcoRI oligonucleotide adapter to give pJA97.

Test of Genes in Cos Cells

The chimaeric F(ab') gene in pJA97 was expressed in COS cells in conjunction with a suitable construct capable of expressing of chimaeric light chain polypeptide as described above. PAGE analysis of the expression products and subsequent inspection of the DNA sequence of the CH1-hinge intron suggested that splicing out of the intron was not occurring correctly leading to the production of an aberrant heavy chain polypeptide.

Reconstruction of IgG4 F(ab') Heavy Chain Gene

Assembly of F(ab') Gene

pJA94 described above was derived from pJA93 which in turn was derived from pJA91. This clone was initially an M13tg130 based vector, i.e. an amber phage capable of being used in the efficient gap-heteroduplex mutagenesis procedure described earlier. In order to repeat the mutagenesis procedure at high yield, the chimaeric F(ab') heavy chain gene was isolated as an EcoRI fragment and recloned into M13tg130 to give pJA100. By oligonucleotide directed site specific mutagenesis, a SalI site was introduced towards the 3' end of the

CH1 exon to give pJA108. The introduced SalI site in
the CH1 domain codes for the fifth and fourth from
last amino acids of the CH1 domain. To reconstruct
the hinge into the end of the CH1 domain, four
oligonucleotides were made which together are able to
code for the last five amino acids of the CH1 domain,
the hinge sequence, two in-frame stop codons and an
EcoRI site.

The oligonucleotides were assembled and cloned
into M13 and mp11 between the SalI and EcoRI sites in
the polylinker, sequenced, reisolated and ligated to
the gene containing the EcoRI-SalI 700bp fragment
from pJA108 to reconstruct the chimaeric B72.3 F(ab')
heavy chain gene.

The reconstructed CH1/hinge sequence should be:

CH1   hinge

Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro
Ser Cys Pro Stop

The oligonucleotide used to form this CH1/hinge were

1.   5TCGACAAGAGAGTTGAGTCCAAATATGGG
2.   3'GTTCTCTCAACTCAGGTTTATACCCGGGGG

3.   5'CCCCCGTGCCCATCATGCCCATGATG
4.   3'CACGGGTAGTACGGGTACTACTTAA

In the vector, oligonucleotides 1 and 3 produced the
sense strand and oligonucleotides 2 and 4 produced
the anti-sense strand.

<u>Assembly of Gene in Expression Vector</u>

The chimaeric B72.3 F(ab') heavy chain gene
fragment, assembled as described above, was
subsequently cloned into the EcoRI vector fragment of
pJA96 to give pJA114.

<u>Test of Genes in COS Cells</u>

The genes were tested in COS cells as described
above. On non-reducing SDS-PAGE the material
appeared to be produced as F(ab') material only.
Reducing SDS-PAGE showed the presence of light chain
and truncated heavy chain equivalent to that expected
from the F(ab') gene.

<u>Development of Stable Cell Lines in CHO Cells</u>

The expression plasmid pJA114, comprising the
B72.3 chimaeric F(ab') heavy chain gene fragment
expressed from the HCMV promoter, was introduced by
electroporation into the CHO cell line CL18 described
above. The procedure was similar to that described
for introduction of the full length chimaeric heavy
chain except that the SalI digestion was omitted and
the DNA was introduced as closed circular DNA. Cell
lines resistant to mycophenolic acid and expressing
function F(ab') antibody were identified by screening
culture supernatants in an antigen binding ELISA
format assay as described earlier. Cell lines
expressing between 0.1-6 µg/ml F(ab') were isolated.
One cell line, FB9 was used for further studies.

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Purification of Chimaeric F(ab')₂   Antibody

Chimaeric F(ab') was purified from CHO cell
supernatant using immunopurification. An
immunopurification reagent was prepared by linking
NH3/41, an antibody was specificity for human Kappa
chain sequence, to cyanogen bromide activated
Sepharose by standard methodology. This material was
packed into a column and equilibrated with PBS. CHO
cell culture supernatant containing chimaeric F(ab')
was applied to the column and the column was washed
with PBS. Elution of chimaeric F(ab') was then
achieved using 4.5M guanidine hydrochloride.
Fractions containing chimaeric F(ab') were then
dialyzed extensively into PBS and concentrated by
ultrafiltration.

Purity and assembly of F(ab') was tested by
SDS-PAGE (both reducing and nonreducing and by HPLC
gel filtration. Antigen binding activity was
demonstrated using an ELISA format assay.
Approximately 10% of the material can be found as
F(ab')₂   which forms without further treatment.

EXAMPLE 4

Chimaeric B72.3 IgG1 F(ab')₂

Construction of F(ab') Heavy Chain Gene

Example 2 discloses the vector RB22 which
contains the B72.3/human IgG1 chimaeric cloned gene
in pAT153. Vectors JA96 and JA108 are mentioned
above. The plasmid TR002 containing hinge modified
gene was constructed as shown in Figure 13. The

chimaeric F(ab') region containing the B72.3 VH/IgG1 was isolated as a 0.7 kbp fragment from RB22 by treating the DNA with SalI, removing the 5' phosphate from the SalI site with calf intestinal phosphatase (CIP) and recutting the DNA with EcoRI.

The IgG1 hinge was assembled by kinase labelling 500pm of top and bottom strand oligonucleotide and annealing the oligonucleotides by heating to 70°C and cooling to room temperature in the kinase buffer. The hinge fragments were ligated to the 0.7 kbp fragment from JA108 prepared as above, and the CIP'ed 5' ends were kinased.

Assembly of Gene in Expression Vector

The chimaeric B72.3 IgG1 F(ab') heavy chain gene fragment, assembled as described above, was subsequently cloned into the EcorI/CIP treated vector fragment of JA96 to give TR002. Expression of TR002 in suitable cells with an expression vector capable of producing a useful light chain, for example chimaeric or humanised B72.3 will produce material which should assemble to give F(ab') and which will on suitable post translational modification in vivo or in vitro give F(ab')₂.

Thus, it has been demonstrated that it is possible to produce a HAM having specificity derived from a mouse MAb but having human constant regions, and which may have an important note to play in cancer diagnosis and therapy.

It will be appreciated that the present invention has been described above by way of illustration only, and that variations or modifications of detail can be made without departing from the scope of the invention.

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CLAIMS

1.   A humanised antibody molecule (HAM) having specificity for the TAG-72 antigen and having an antigen binding site wherein at least the complementarity determining regions (CDRs) of the variable domains are derived from the mouse monoclonal antibody B72.3 (B72.3 MAb) and the remaining immunoglobulin-derived parts of the HAM are derived from a human immunoglobulin.

2.   The HAM of claim 1, wherein the entire variable domains are derived from the B72.3 MAb.

3.   The HAM of claim 1 or claim 2, when produced by recombinant DNA technology.

4.   The HAM of any one claims 1 to 3, which comprises a complete antibody molecule, an Fab fragment or an (Fab')$_2$ fragment.

5.   The HAM of any one of claims 1 to 4, wherein an effector or reporter molecule is attached thereto.

6.   The HAM of claim 5, wherein the effector or reporter molecule is a protein molecule which is coexpressed as a fusion protein with one of the chains of the HAM.

7.   A process for producing the HAM of any one of claims 1 to 6, which process comprises

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy or light chain wherein at least the

CDRs of the variable domain are derived from the
B72.3 MAb and the remaining immunoglobulin-derived
parts of the antibody chain are derived from a human
immunoglobulin;

(b) producing in an expression vector an
operon having a DNA sequence which encodes a
complementary antibody light or heavy chain wherein
at least the CDRs of the variable domain are derived
from the B72.3 MAb and the remaining
immunoglobulin-derived parts of the antibody chain
are derived from a human immunoglobulin;

(c) transfecting a host cell with the or each
vector; and

(d) culturing the transfected cell line to
produce the HAM.

8.   The process of claim 7, wherein the heavy and
light chain encoding sequences are present on the
same vector.

9.   The process of claim 7, wherein the heavy and
light chain encoding sequences are present on
separate vectors.

10.   The process of any one of claims 7 to 9,
wherein the DNA coding sequences comprise fusions of
cDNA and genomic DNA.

11.   The process of claim 10, wherein the host cell
is a non-myeloid mammalian cell.

FIG. 1

A

```
        10                30                50
GAATTCCCACTGACTCTAACCATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTA
                     MetGluTrpSerTrpValPheLeuPhePheLeuSerVal

        70          ▽      90                110
ACTACAGGTGTCCACTCCCAGGTTCAGCTGCAGCAGTCTGACGCTGAGTTGGTGAAACCT
ThrThrGlyValHisSerGlnValGlnLeuGlnGlnSerAspAlaGluLeuValLysPro

        130               150               170
GGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGCTACACCTTCACTGACCATGCTATT
GlyAlaSerValLysIleSerCysLysAlaSerGlyTyrThrPheThrAspHisAlaIle

        190               210               230
CACTGGGCGAAGCAGAAGCCTGAACAGGGCCTGGAATGGATTGGATATATTTCTCCCGGA
HisTrpAlaLysGlnLysProGluGlnGlyLeuGluTrpIleGlyTyrIleSerProGly

        250              270               290
AATGATGATATTAAGTACAATGAGAAGTTCAAGGGCAAGGCCACACTGACTGCAGACAAA
AsnAspAspIleLysTyrAsnGluLysPheLysGlyLysAlaThrLeuThrAlaAspLys

        310               330               350
TCCTCCAGCACTGCCTACATGCAGCTCAACAGCCTGACATCTGAGGATTCTGCAGTGTAT
SerSerSerThrAlaTyrMetGlnLeuAsnSerLeuThrSerGluAspSerAlaValTyr

        370               390               410        ▼
TTCTGTAAAAGATCGTACTACGGCCACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
PheCysLysArgSerTyrTyrGlyHisTrpGlyGlnGlyThrThrLeuThrValSerSer
```

B

```
        10                30                50
ATCACACACACACACATGAGTGTGCCCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTT
                  MetSerValProThrGlnValLeuGlyLeuLeuLeuLeuTrpLeu

        70          ▽      90                110
ACAGATGCCAGATGTGACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGTATCTGTG
ThrAspAlaArgCysAspIleGlnMetThrGlnSerProAlaSerLeuSerValSerVal

        130               150               170
GGAGAAACTGTCACCATCACATGTCGAGCAAGTGAGAATATTTACAGTAATTTAGCATGG
GlyGluThrValThrIleThrCysArgAlaSerGluAsnIleTyrSerAsnLeuAlaTrp

        190               210               230
TATCAACAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTATGCTGCAACAAACTTAGCA
TyrGlnGlnLysGlnGlyLysSerProGlnLeuLeuValTyrAlaAlaThrAsnLeuAla

        250               270               290
GATGGTGTGCCATCAAGGTTCAGTGGCAGTGGATCGGGCACACAGTATTCCCTCAAGATC
AspGlyValProSerArgPheSerGlySerGlySerGlyThrGlnTyrSerLeuLysIle

        310               330               350
AACAGCCTGCAGTCTGAAGATTTTGGGAGTTATTACTGTCAACATTTTTGGGGTACTCCG
AsnSerLeuGlnSerGluAspPheGlySerTyrTyrCysGlnHisPheTrpGlyThrPro

        370               390               410      ▼
TACACGTTCGGAGGGGGGACCAGGCTGGAAATAAAACGGGCTGATGCTGCACCAACTGTC
TyrThrPheGlyGlyGlyThrArgLeuGluIleLysArgAlaAspAlaAlaProThrVal
```

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FIG. 2

Chimaeric heavy chain gene
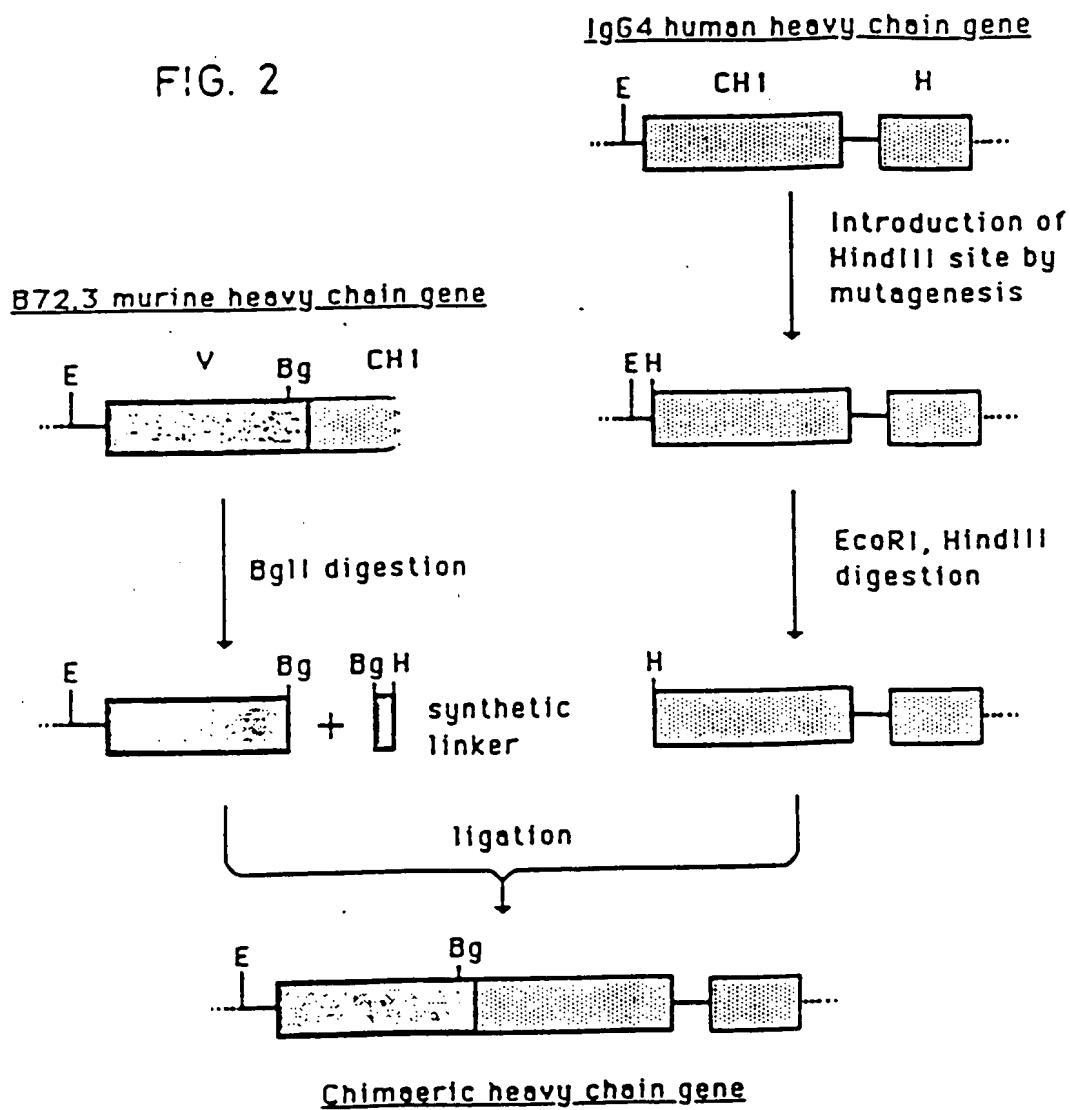
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B72.3 murine light chain gene
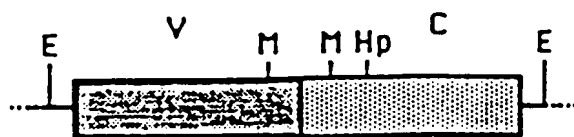
FIG. 3

EcoRI, Hpa I
digestion
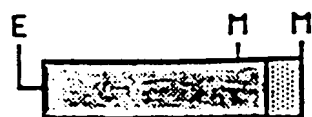
Human K light chain gene

Mboll partial
digestion
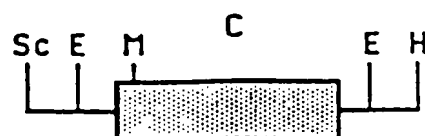
Mboll
digestion

ligation
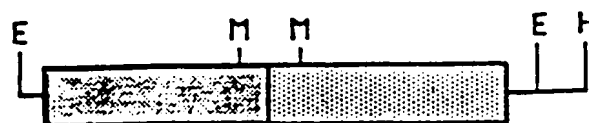
Chimaeric light chain gene

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1   Murine heavy chain cDNA

V   CH1 H CH2 CH3

2   Murine light chain cDNA

V   C   FIG. 4
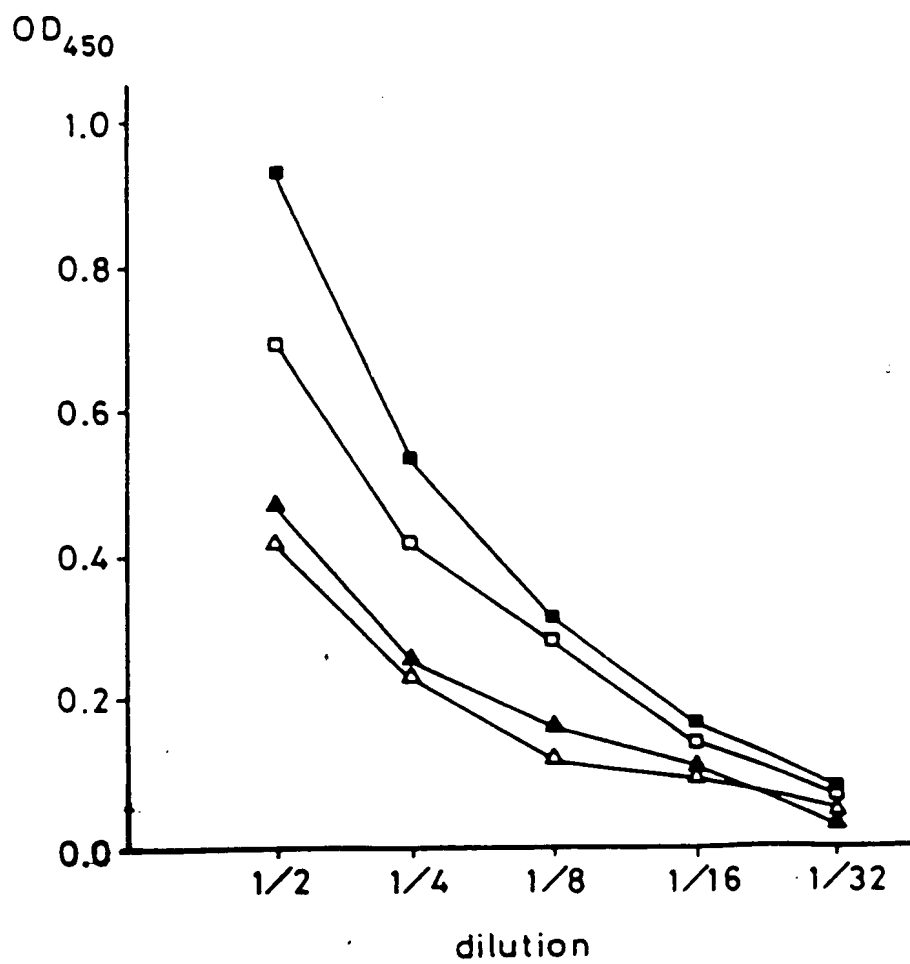
3   Chimaeric heavy chain gene
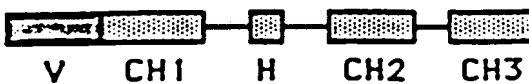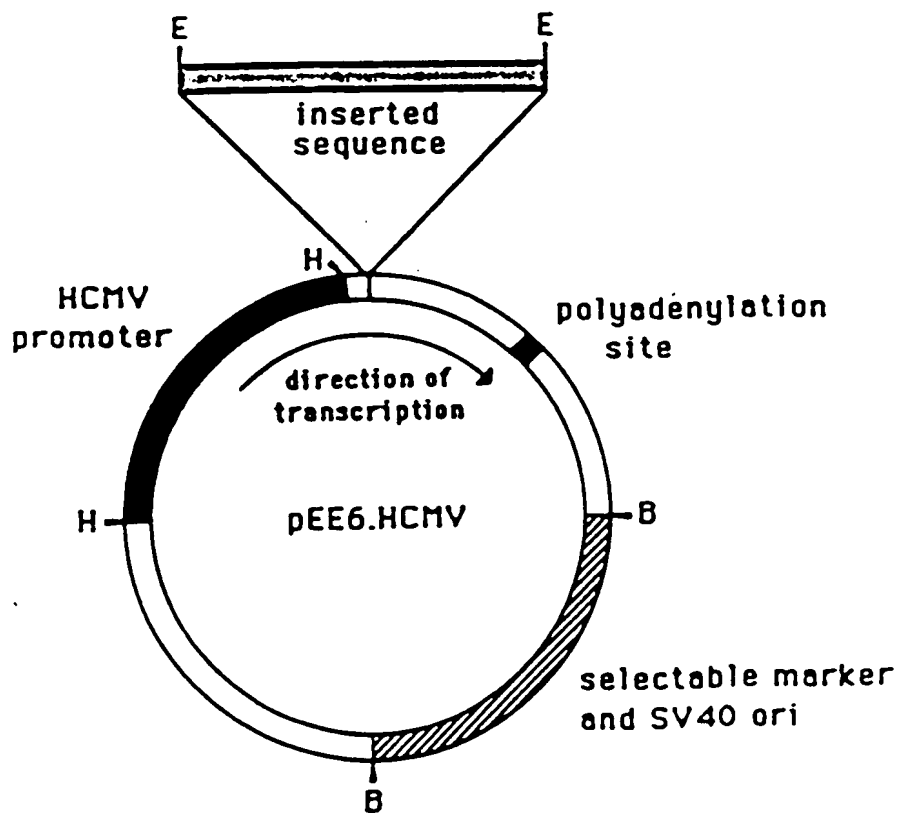
V   CH1   H   CH2   CH3

4   Chimaeric light chain gene

V   C

FIG. 5

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1   2   3   4   5   6

- 94

- 67

γ -   ▬ ▬   ▬ ▬   - 43

- 30

κ -   ▬ ▬ ▬ ▬ ▬ ▬

- 20

FIG. 6

Non-red.   Red.
1   2   3   4   5   6

— —   - 150

                          - 94

                          - 67

          — —

                          - 43

                          - 30

      — — —

  —

                          - 20

FIG. 7

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FIG. 8

SDS-PAGE OF CHIMAERIC B72.3

NON-REDUCING   REDUCING

94K▶

67K▶

94K▶

43K▶

30K▶

67K▶

20K▶
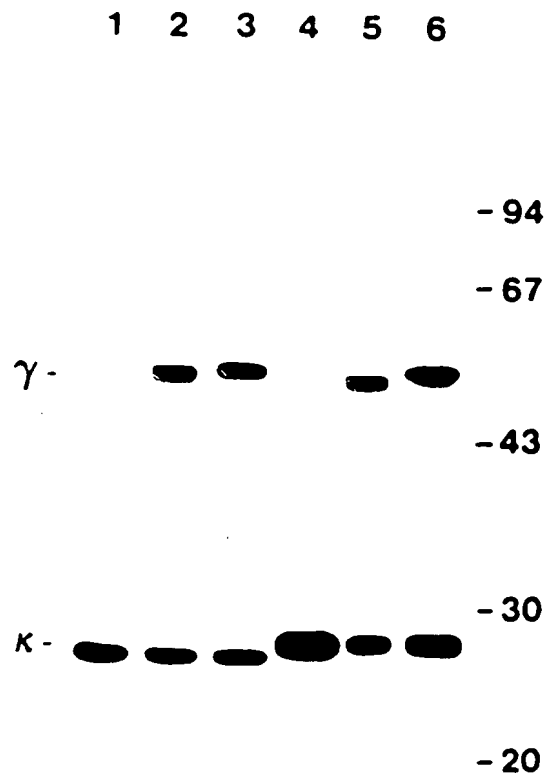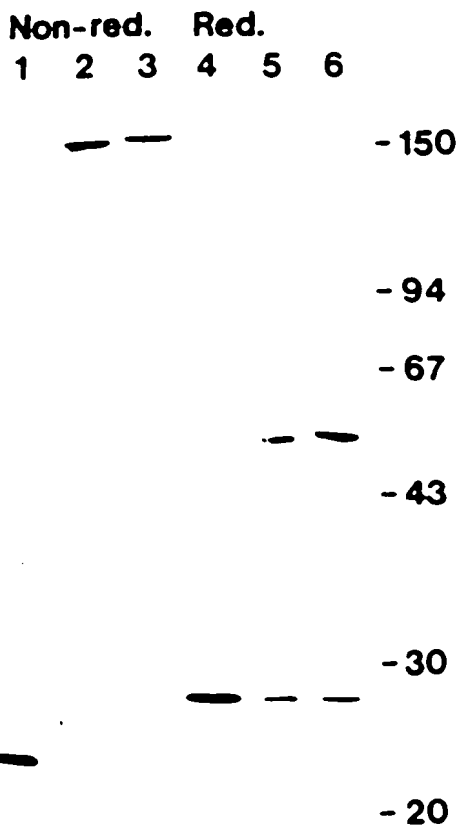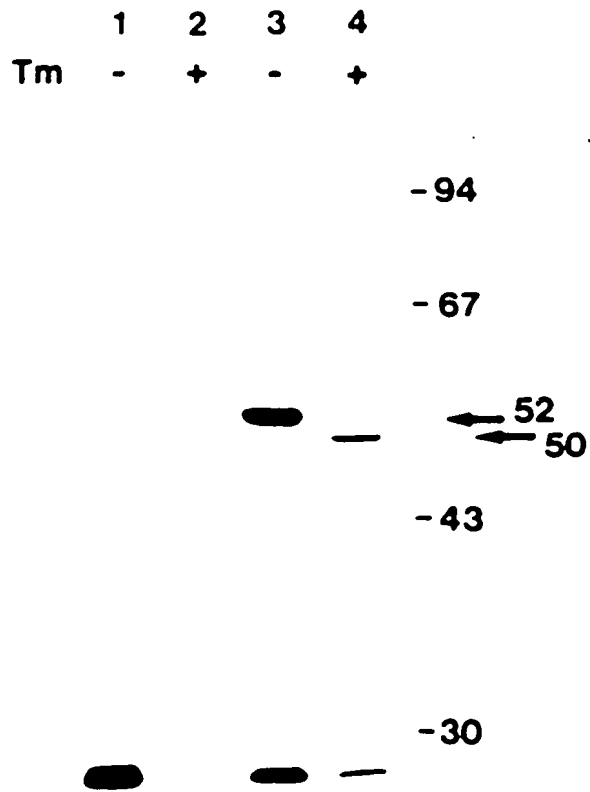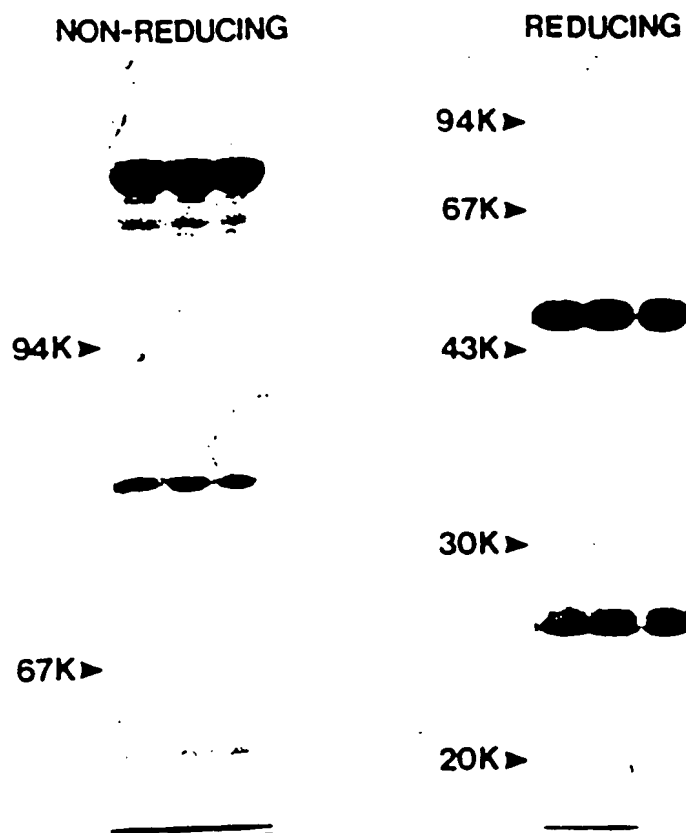
FIG. 9

2-DIMENSIONAL SDS-PAGE OF CHIMAERIC B72.3
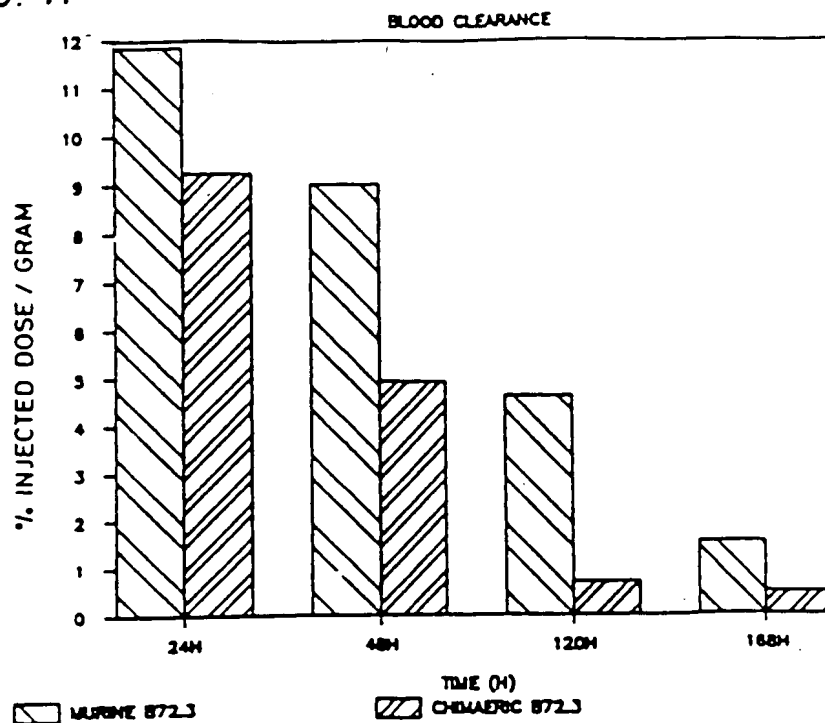
⊢———▶ decreasing Mr of non-reducing gel
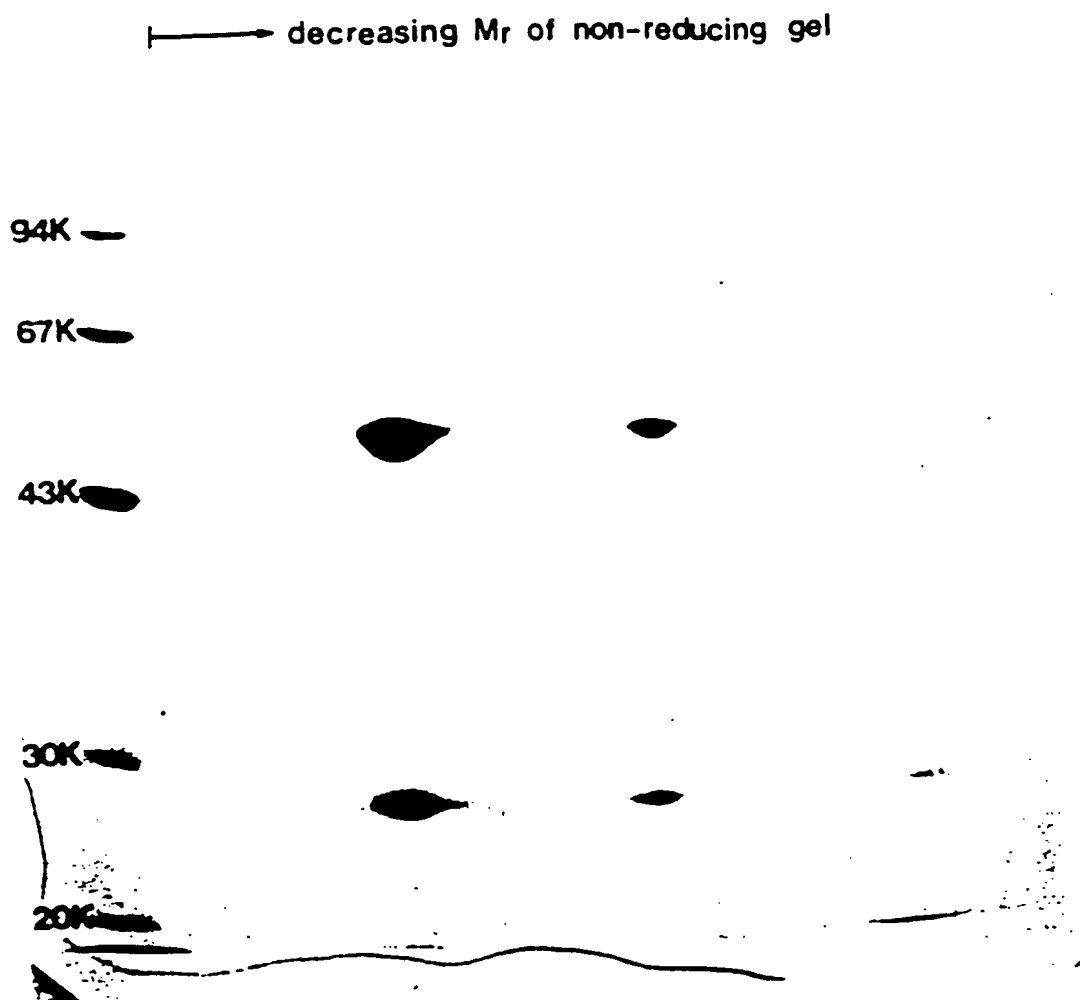
1st dimension : non-reducing SDS-PAGE
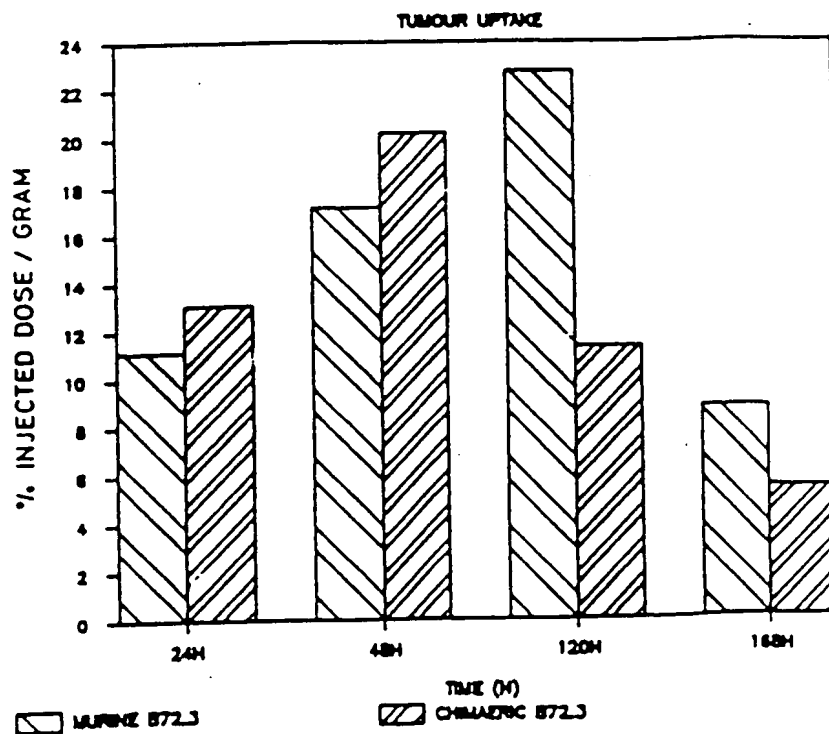2nd dimension : reducing SDS-PAGE

FIG. 10

FIG. 11   LS174T XENOGRAFT: CHIMAERIC B72.3

LS174T XENOGRAFT: CHIMAERIC B72.3

FIG. 12

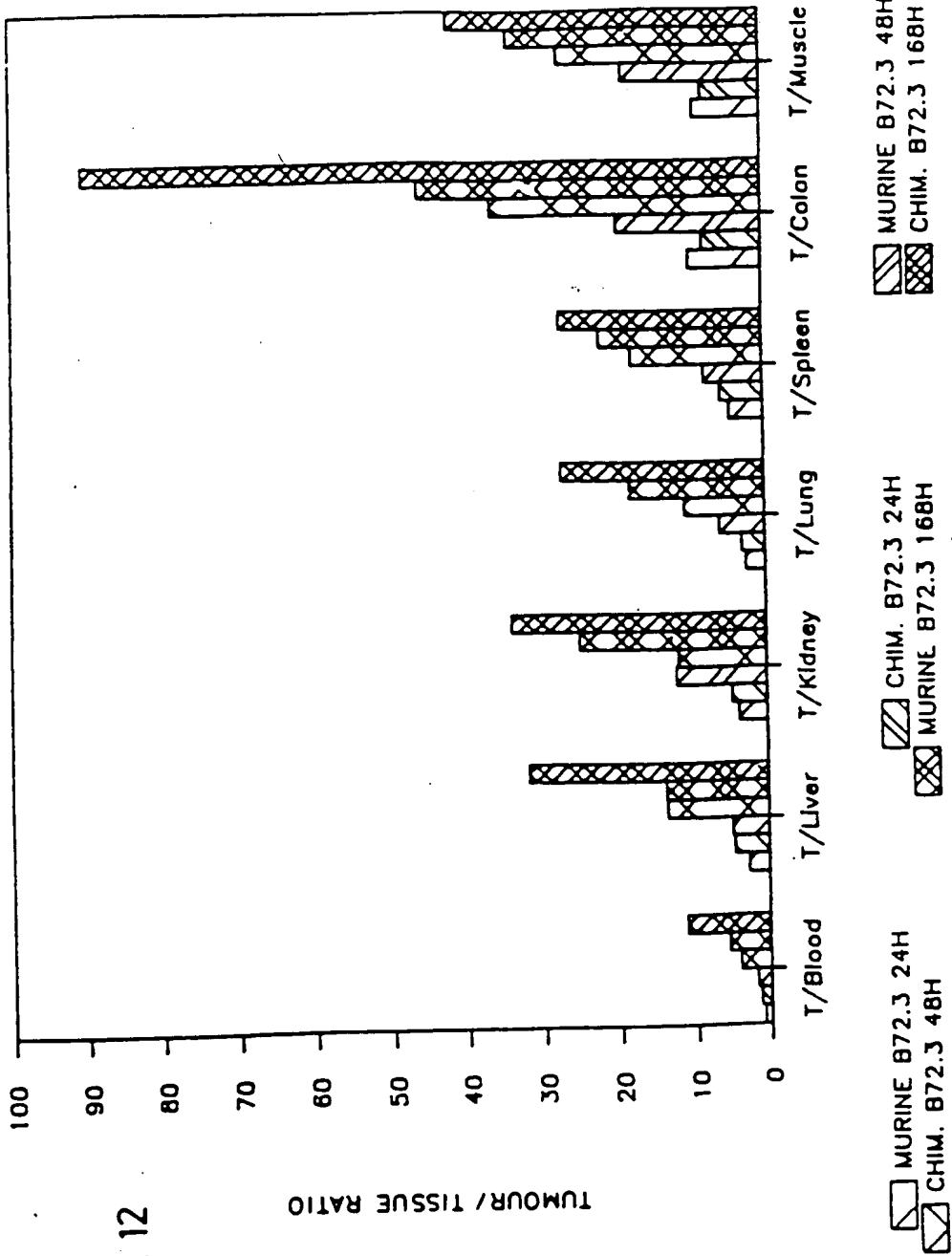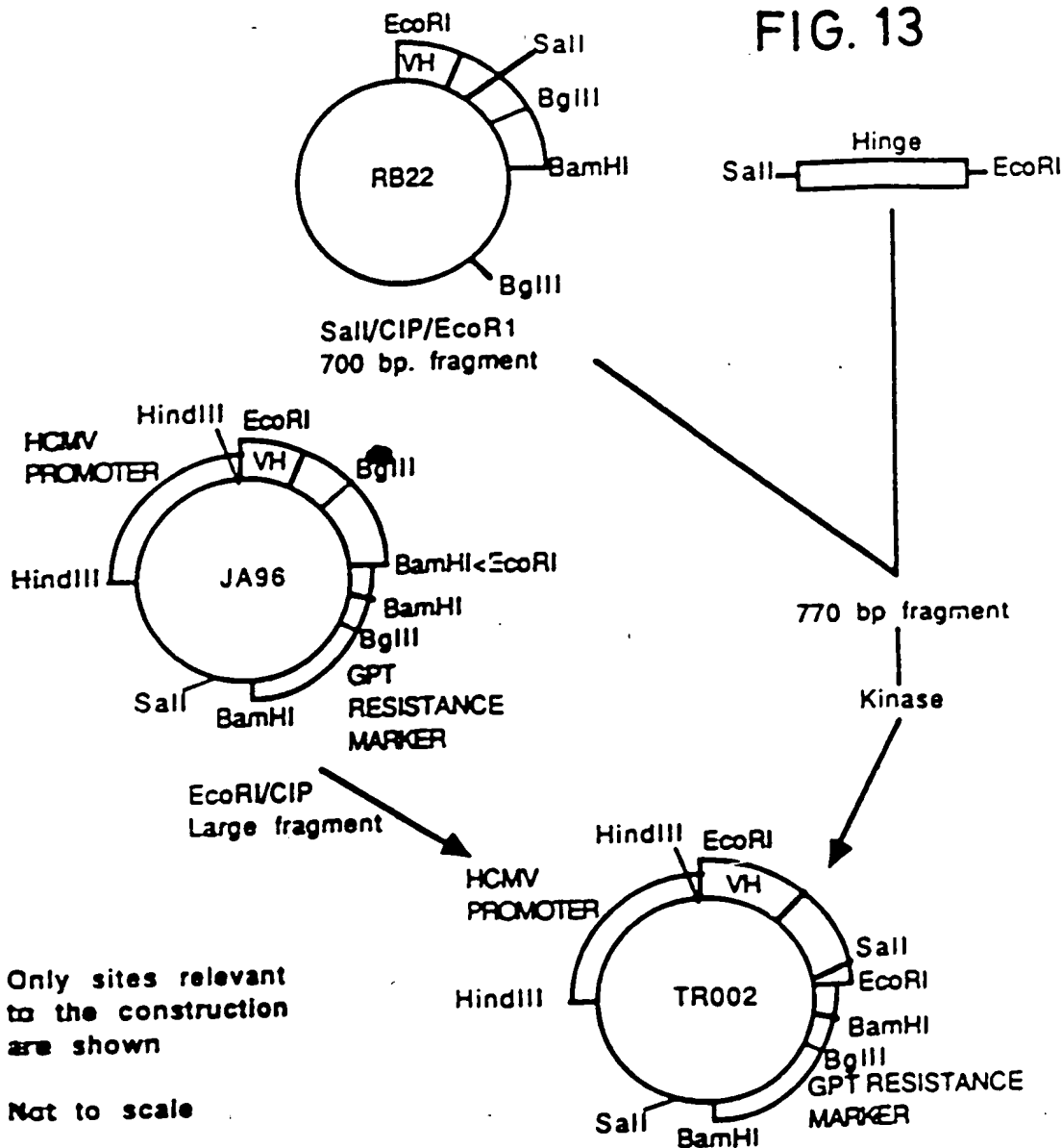CHIMAERIC B72.3 v MURINE B72.3

TUMOUR/TISSUE RATIOS:24—168H

TUMOUR/TISSUE RATIO

T/Blood   T/Liver   T/Kidney   T/Lung   T/Spleen   T/Colon   T/Muscle

MURINE B72.3 24H   CHIM. B72.3 24H   MURINE B72.3 48H
CHIM. B72.3 48H   MURINE B72.3 168H   CHIM. B72.3 168H

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FIG. 13

CONSTRUCTION OF TR002
B72.3VH/HUMAN IgG1 F(ab') CONSTANT
cDNA LIKE CHIMAERIC HEAVY CHAIN (ɣ1 HINGE)
IN EE6 BASED EXPRESSION VECTOR